Synopsis

Synthesis, Characterization and Computational studies of Schiff bases from Salicylaldehyde and its derivatives with their metal complexes

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Introduction

The Schiff bases are the compounds prepared by a basic condensation process between ketones or aldehydes with various amines, where an azomethine group has superseded the carbonyl group. The various role of Schiff base ligands and their metal complexes in biochemistry, inorganic, and metal-organic have received substantial attention due to their extensive applications in a wide range of areas. Schiff base metal complexes show various optical, magnetic, and chemical properties by modifying the different ligands (**Sahar, S. et al.**). The Schiff bases and metal complexes are powerful chelating agents because their azomethine nitrogen atoms have high electron density. The Schiff base ligands and their metal complexes have a wide range of applications in various fields such as analytical, catalysts, clinical, corrosion inhibitors, intermediates, dyes, medicine, polymer stabilizers, agrochemicals, pharmacology and etc. (**Nora, S. A. et al.**).

4-amino antipyrine (4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one) is a pyrazolone derivative (**Subbaiyan, S. et al.**), which is the transformation product of aminopyrine, 4-acetamido antipyrine, and 4-formyl amino antipyrine. It is a useful chromogenic reagent in determining various phenols in an aqueous solution for biochemical reactions producing phenols or peroxides. 4-amino antipyrine stimulates liver microsomes and is used to measure extracellular water. 4-amino antipyrine has shown various pharmacological activities such as anti-inflammatory, antipyretic, analgesic, antioxidant, and antimicrobial properties (**Qazi, Y. et al.**).

Salicylaldehyde is an aromatic organic compound prepared by heating phenol and chloroform with sodium hydroxide or potassium hydroxide in a Reimer-Tiemann reaction (**Yiying, Y. et al.,**). Salicylaldehyde has naturally occurred in Spireaulmaria flowers, Crepisfoetida L. root, Pinusavium fruit, Rauqolfiacaffra rind, Ceanothusvelutinus leaf, Cinnamomum cassia, tobacco leaves, grapes, baked potato, tomato, cinnamon bark, peppermint oil, cassia leaf, pennyroyal oil, butter, parmesan cheese, milk powder, roasted chicken, sherry, soybean, coffee, tea, mushroom, buckwheat, Muscat grape, Chinese quince, and mastic gum oil.

Salicylaldehyde and its derivatives are mainly used in perfumes, fumigants, flavor ingredients in foods, and medicinal chemicals, which are also used to find ketonuria in treatment, and colorimetric analysis water. Salicylaldehyde and 4-amino antipyrine-based Schiff base compounds and their metal complexes have various biological applications: antitumor, antimicrobial, anti-malarial, etc. (**Lan-Qin, C. et al.,**).

Ortho-vanillin Schiff base is synthesized from the condensation of 2-Hydroxy-salicylaldehyde with various amines to produce potential ligands. The metal complexes of ortho-vanillin Schiff base ligands are highly stable due to their strong coordination, having various applications in biological, pharmaceuticals, clinical and industrial fields. The ortho-vanillin is used as an antioxidant, anti-carcinogenic, free radical scavenging, antimutagenic and antimicrobial agent (**Costes, J., P., et al.,**).

5-chloro salicylaldehyde is a useful synthetic intermediate and an essential precursor for various chelating agents. 5-chloro salicylaldehyde Schiff bases and their metal complexes have potential biological activities (**Lei, S. et al.,**).

5-bromo salicylaldehyde is a derivative of salicylaldehyde, which has potential biological activities. 5-bromo salicylaldehyde is an important reagent to synthesis diaryl amino-substituted N-methyl tetra hydro salen. The main application of the 5-bromo salicylaldehyde is used for chemical derivation during amine quantification in poly(ethylene terephthalate) (PET) film and PET scaffold (**Oteyaka, M., O., et al.,**).

Objectives of the study

Ø Synthesis of Schiff bases derived from 4-amino antipyrine with salicylaldehyde and its derivatives.

Ø Synthesis of Schiff base complexes derived from Schiff base ligands with CuCl2(PPh3)2, CoCl2(PPh3)2, and NiCl2(PPh3)2 complexes.

Ø Characterization of synthesized Schiff bases and their metal complexes using various Physico-chemical techniques, such as elemental analysis (CHN), FT-IR, 1H NMR, 13C NMR, 31P NMR,

ESR, GC-MS, LC-MS, and the structure of Schiff bases and their metal complexes are optimized using Gaussian software.

Ø To carry out Molecular docking studies for the newly synthesized Schiff bases and their metal complexes against COVID-19 and activate T cells.

Experimental Section

Phase I: Materials

Metal salts (CoCl2.6H2O, CuCl2.6H2O, NiCl2.6H2O) were purchased from Himedia, Triphenylphosphine, and 4-amino antipyrine were purchased from Sigma Aldrich, Salicylaldehyde, o-Vanillin, 5-chloro salicylaldehyde, 5-bromo salicylaldehyde were purchased from Loba Chemie, and the solvents were purchased from Merck. All the chemicals were purchased in analytical reagent grade.

Physical measurements

The digital melting point apparatus (SAFIRE) was used to determine the melting point of the synthesized Schiff base ligands and their metal complexes using an open capillary tube at a heating rate of 10oC/min. Percentage of the Carbon, Hydrogen, and Nitrogen were measured by using Thermal Finnigan Elemental Micro Analyzer. The Fourier-transform infrared spectra of the synthesized Schiff base ligands and their metal complexes were recorded on the JASCO FT/IR 4700 spectrometer.1H NMR (400 MHz), 13C NMR (100 MHz), and 31P NMR (162 MHz) spectra were recorded on a BRUKER ADVANCE III HD NANOBAY 400 MHZ FT-NMR Spectrometer in CDCl3 using TMS as an internal standard. The gas chromatography-mass spectrum of the Schiff base ligands was recorded by using the AGILENT instrument, and the liquid chromatography-mass spectra of the Schiff base complexes were recorded in VARIAN Inc, USA (410 Prostar Binary LC with 500 MS IT PDA Detectors) instrument. The electron spin resonance spectra of synthesized Schiff base complexes were recorded by using a JEOL spectrometer. The thin layer chromatography was used to monitor the reactions of the Schiff base ligands and their metal complexes.

Phase II: Methods

General procedure to synthesize Schiff bases

Equimolar mixtures of amine and various aldehydes were refluxed with alcohol over a water bath for about 3 hours were monitored by thin-layer chromatography. The resulted products were separated and dried under vacuum.

The synthesized four Schiff base ligands are namely SAP, OVAP, SAPCl, and SAPBr. SAP has been prepared from 4-amino antipyrine with Salicylaldehyde, OVAP has been prepared from 4-amino antipyrine with 3-methoxy-salicylaldehyde, SAPCl has been prepared from 4-amino antipyrine with 5-chloro salicylaldehyde, and SAPBr has been prepared from 4-amino antipyrine with 5-bromo salicylaldehyde,

General procedure to synthesize Schiff base complexes

Equimolar mixtures of synthesized Schiff base ligand and metal precursor complexes of the type $[MCl_2(PPh_3)_2]$ (M=Cu(II), Co(II), Ni(II)) were refluxed with alcohol over a water bath for about 6 hours were monitored by thin-layer chromatography. The resulted products were separated and dried under vacuum.

Density Functional Theory

The molecular structures were drawn and visualized using Avogadro software. The geometry of the synthesized Schiff base ligands and their metal complexes were optimized using density functional theory by hybrid function B3LYP (Becke 3-parameter Lee-Yang-Parr) with the base set 6-31+ G (d, p) calculation. After completing the process, the theoretical nuclear magnetic resonance of synthesized Schiff base ligands and their metal complexes were recorded using 6-31+ G (d, p)/B3LYP with the GIAO method. The optimization method has been done by Gaussian 09w and Gauss view 6.0.1 software. The energetic behavior of the synthesized Schiff base ligands and their metal complexes were carried out to be evaluated by HOMO-LUMO energy calculations.

The Docking Study

The optimized structure of the Schiff base ligands and their metal complexes were converted into the Sybyl.mol2 file using Gaussian software, and structures utilized for the molecular docking studies against Coronavirus main protease (6lu7) and T-cell activation (TCR-MHC-like molecule - 6bnk)using Auto Dock 4.2, Auto Dock Tools 1.5.6, and Auto Dock Vina 1.1.2 software. The Coronavirus main protease and the TCR-MHC-like molecule were downloaded from Research Collaboratory for Structural Bioinformatics Protein Data Bank.

The ADME Study

Drug development aims to convert compounds with therapeutic effects into drug forms that can be done to patients. The injected drug must reach the target (site of action), exerts pharmacological effects, and be eliminated within a reasonable time frame to allow once per day dosing. So, the determination of the viability of a drug candidate is essential. The ADME study helps to determine the viability of a drug. The abbreviated form of ADME study is absorption (How much of the drug is absorbed and quickly), distribution (Where is the drug distributed within the body, the rate, and extent of the distribution), metabolism (How fast is the drug metabolized and the mechanism of action), elimination (How is the medicine excreted). The computational ADME studies were carried out for the Schiff base ligands and their metal complexes using SwissADME.

Results and Discussion

Phase I: Characterization of synthesized Schiff base ligands and their metal complexes

The experimental and simulated FT-IR analysis

Infrared spectroscopic comparison and study of Schiff base ligand and its metal complexes shows that the ligand is dentate with azomethine nitrogen, carbonyl oxygen, and phenolic oxygen as three coordination sites. A peak of the azomethine (C=N) group around 1650 cm-1 is observed in the Schiff bases experimental and simulated infrared spectra. The experimental and simulated infrared spectra observed carbonyl group (C=O) of the 4-amino antipyrine moiety in the Schiff base ligands at 1750 to 1755 cm-1. Weak broadband has appeared

in the experimental infrared spectra and simulated infrared spectra at 3505 to 3510 cm-1, indicating phenolic –OH of the salicylaldehyde ring moiety. In the Schiff base complexes, azomethine (C=N) and carbonyl (C=O) bands are observed lower wavenumbers due to the azomethine and carbonyl groups coordinated with the metal center. The experimental and simulated infrared spectra of the Schiff base complexes observed the azomethine (C=N) peak at 1590 to 1600 cm-1. As well as the carbonyl (C=O) peak appeared at 1650 to 1620 cm-1 in the experimental and simulated infrared spectra. Important phenolic -OH peak disappeared due to the bond formation between phenolic oxygen with the central metal atom (M-O). The M-O bond has observed a peak at 490 to 505 cm-1 in the experimental and simulated infrared spectra of the Schiff base complexes. Another important peak of the Schiff base complexes observed the metal-nitrogen (M-N) band in the experimental and simulated infrared spectra at 430 to 440 cm-1.

The experimental and simulated 1H NMR analysis

In the synthesized Schiff base ligands, the experimental and simulated azomethine proton (CH=N) observed the chemical shift value at 8.5 to 10 ppm. The singlet chemical shift value occurred at 13.00 to 13.50 ppm in the experimental and theoretical proton NMR spectra, which corresponds to the phenolic -OH proton. The azomethine proton (CH=N) of the synthesized Schiff base complexes appears in the experimental and simulated proton NMR spectra at 7.8 to 8.10 ppm. The phenolic –OH chemical shift was disappeared in the NMR spectra of Schiff base complexes due to the formation of the M-O bond.

The experimental and simulated 13C NMR analysis

The two important chemical shifts of the synthesized Schiff base ligands are appeared nearly in both experimental and the simulated 13C NMR spectra at around 160 ppm (HC=N) and C=O around 161 ppm (C=O). However, the azomethine and carbonyl carbons of the synthesized Schiff base complexes observed the peaks at below 159 ppm (HC=N), approximately 160.70 ppm (C=O) in the experimental and theoretical 13C NMR spectrum. Here the azomethine and carbonyl are observed lower chemical shift values due to the coordination of ligand with the central metal atom.

Frontier molecular orbital analysis

The frontier molecular orbital analysis is used to discover the electric and optical properties of the compounds or complexes. The frontier molecular orbital energy gaps are calculated using the B3LYP/6-31+G(d,p) method in Gaussian software. According to the frontier molecular orbital analysis, the higher energy gap indicates the molecule has low chemical reactivity and high kinetic stability; the lower energy gap expresses that the molecule has high chemical reactivity and low kinetic stability. The energy gap between SAP to SAPNi, SAP has a higher energy gap (EHOMO-LUMO= -0.07411a.u.), and SAPCo has a lower energy gap (EHOMO-LUMO= -0.00205a.u.). The energy gap between OVAP to OVAPNi, OVAP(EHOMO-LUMO= -0.02240a.u.) has the lowest energy gap with high chemical reactivity and low kinetic stability, OVAPNi (EHOMO-LUMO=0.05466 a.u.) has a high energy gap with low chemical reactivity and high kinetic stability due to it is energetically unfavorable to add an electron to LUMO in order to remove electrons from HOMO. The energy gap between SAPCl to SAPClNi, SAPCl has a higher energy gap (EHOMO-LUMO= -0.07411a.u.), and SAPClNi has a lower energy gap (EHOMO-LUMO= -0.00319a.u.). The energy gap between SAPBr to SAPBrNi, SAPBr has a higher energy gap (EHOMO-LUMO= -0.08734 a.u.), and SAPBrNi has a lower energy gap (EHOMO-LUMO= -0.00323a.u.).

Mass spectral study

The synthesized Schiff bases and their complexes observed mass spectral values, 307.02 m/z (SAP), 668.11 m/z (SAPCu), 933.27 m/z (SAPCo), 671.18 m/z (SAPNi), 338 m/z (OVAP), 699.2476 m/z (OVAPCu), 997.490 m/z (OVAPCo), 736.2258 m/z (OVAPNi), 342.12 m/z (SAPCl), 718.28 m/z (SAPClCu), 772.32 m/z (SAPClCo), 738.11 m/z (SAPClNi), 362.92 m/z (SAPBr), 752.04 m/z (SAPBrCu), 739.10 m/z (SAPBrCo), 831.89 m/z (SAPBrNi).

ESR study

The synthesized Schiff base complexes, i.e., SAPCu, SAPNi, OVAPCu, OVAPNi, and SAPClCu, are observed ESR spectra. According to the ESR spectra, SAPCu, OVAPCu, SAPClCu are trigonal bipyramidal structures, and SAPNi, OVAPNi are octahedral structures.

Phase II: Molecular docking studies of the synthesized Schiff base ligands and their metal complexes

TCR-MHC-like molecule (6BNK)

TCR-MHC (6BNK) like molecule has two main parts: Mus musculus and Homo sapiens, each part having four chains. Mus musculus model CD1d1 has A, E chains and B, F chains present in its beta-2-microglobulin and Human nkt TCR alpha chain and Human nkt TCR beta chain has C, G, D, H chains in Homo sapiens model. Recently the T cells are activated by KRN7000, AH15-1, AH03-1, and AH10-7 molecules (**Chennamadhavuni, D. et al.**). So, here we compared our reported Schiff base ligands and their metal complexes with KRN7000, AH15-1, AH03-1, and AH10-7 for Mus musculus and Homo sapiens in 6BNK (TCR-MHC like molecule). The reported Schiff base ligands and their metal complexes have higher negative values than KRN7000, AH15-1, AH03-1, and AH10-7. According to the binding affinity, a higher negative value has higher biological activity. Compared with Schiff base ligands, Schiff base complexes have a strong binding affinity. However, the drug-likeness of the molecules is essential in biological activity. Lipinsky's rule of five provides drug-likeness from the molecule's physical and chemical properties, making it a probable orally active drug in human beings. Lipinsky's rule of five has been carried out to the synthesized Schiff base ligands and their metal complexes. All the reported Schiff base ligands obey Lipinsky's rule of five compared with metal complexes, and each metal complexes have one violation due to its molecular weight.

COVID-19 main protease (6LU7)

In the molecular docking studies against Coronavirus, the reported Schiff base complexes have higher binding affinity compared with broad-spectrum antiviral drug Remdesivir. Also, the drug-likeness was found out by using Lipinsky's rule of five, and here also all the reported Schiff base ligands to obey Lipinsky's rule of five compared with their metal complexes. The theoretical IC50 values and R2 values are calculated from the binding energy and inhibition constant of the .dlg file using auto dock software to each Schiff base ligands and their metal complexes (**Du, X. et al.**).

ADME Study

After the molecular docking study, we are mainly focused on the bioavailability score of the reported Schiff base ligands, and their metal complexes are investigated using SwissADME. Usually, the bioavailability score should be 0.55, which indicates an excellent oral drug (**Kalai, F. E., et al.,**).

Conclusion

Finally, the synthesized Schiff base ligands and their metal complexes are observed related spectral peaks in both experimental and theoretical spectra. The molecular docking of the resulting Schiff base ligands and their metal complexes have higher binding affinity than KRN7000, AH15-1, AH03-1, and AH10-7, as well as Schiff base complexes potential activity against Coronavirus compared with Remdesivir. The reported Schiff base ligands and their metal complexes are observed IC50 value in micromolar concentration. The regression graph R2 value of all reported Schiff base ligands and their metal complexes have the goodness of fit statistic in regression analysis, which means that the reported Schiff base ligands and their metal complexes have more binding energy with higher inhibition constant. SwissADME results of SAP, SAPNi, OVAP, SAPCl, SAPClCo, SAPClNi, SAPBr, SAPBrCo, SAPBrNi have exactly 0.55 bioavailability scores, so these are good oral drugs.

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Publications

1. Designing a new vanillin Schiff base (Z)-4-((2-hydroxy-3-methoxy benzylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one: Synthesis, characterization, crystal structure and biological studies, M, Gowri ; B, Saranya ; S, Athimoolam, Indian Journal of Chemistry Vol. 60B, August 2021, pp. 1110-1116.
2. Synthesis, Characterization, DFT study and Molecular Docking of (Z)-4-((2-hydroxy-3-methoxy benzylidene)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one and its

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